PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 14 March 2001 (14.03.01)	
International application No. PCT/US00/02688	**Applicant's or agent's file reference** 74/82
International filing date (day/month/year) 04 February 2000 (04.02.00)	**Priority date** (day/month/year) 04 February 1999 (04.02.99)
Applicant MERCHAV, Shoshana et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

31 August 2000 (31.08.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Henrik Nyberg

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREAT

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING
AMENDMENTS OF THE CLAIMS

(PCT Rule 62 and
Administrative Instructions, Section 417)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as International Preliminary Examining Authority

Date of mailing (day/month/year)	
14 March 2001 (14.03.01)	
International application No.	**International filing date (day/month/year)**
PCT/US00/02688	04 February 2000 (04.02.00)

Applicant

TECHNION RESEARCH & DEVELOPMENT FOUNDATION LTD. et al

The International Bureau hereby informs the International Preliminary Examining Authority that no amendments under Article 19 have been received by the International Bureau (Administrative Instructions, Section 417).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740 14 35

Authorized officer

Henrik Nyberg

Telephone No. (41-22) 338 83 38

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/02688

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12N 5/00, 5/06, 11/02, 11/08; A61F 2/02; C12M 3/00, 3/04
US CL :424/93.7, 423; 435/177, 180, 347, 373, 395, 398, 289.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.7, 423; 435/177, 180, 347, 373, 395, 398, 289.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST (U.S. PATENTS)
search terms: stem cells, continuous, plug-flow bioreactor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,541,107 A (NAUGHTON et al.) 30 July 1996, entire document, especially column 6, line 36; column 15, lines 41 and 57; column 21, lines 3, 9 and 26.	1-88
Y --- X	US 5,266,476 A (SUSSMAN et al.) 30 November 1993, entire document.	1-88 --- 89-99
Y	US 5,510,262 A (STEPHANOPOULOS et al.) 23 April 1996, entire document.	1-88
Y	US 5,437,994 A (EMERSON et al.) 01 August 1995, entire document.	21-50, 71-88

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 10 MAY 2000	Date of mailing of the international search report 20 JUN 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DAVID M. NAFF Telephone No. (703) 308-0196

JOYCE BRIDGERS
PARALEGAL SPECIALIST
CHEMICAL MATRIX

Form PCT/ISA/210 (second sheet) (July 1998)★

09/890401

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 00/20187	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US00/02688	International filing date *(day/month/year)* 04 FEBRUARY 2000	Priority date *(day/month/year)* 04 FEBRUARY 1999
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant TECHNION RESEARCH & DEVELOPMENT FOUNDATION LTD.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

   - I [X] Basis of the report
   - II [ ] Priority
   - III [ ] Non-establishment of report with regard to novelty, inventive step or industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI [ ] Certain documents cited
   - VII [ ] Certain defects in the international application
   - VIII [ ] Certain observations on the international application

Date of submission of the demand	Date of completion of this report
31 AUGUST 2000	25 JULY 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DAVID M. NAFF Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (July 1998)*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/02688

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the claims:
pages (See Attached) , as originally filed
pages _____ , as amended (together with any statement) under Article 19
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the drawings:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the sequence listing part of the description:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

☒ the description, pages NONE

☒ the claims, Nos. 89-99

☒ the drawings, sheets/~~fig~~ NONE

5. ☐ This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/02688

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	1-88	YES
	Claims	NONE	NO
Inventive Step (IS)	Claims	NONE	YES
	Claims	1-88	NO
Industrial Applicability (IA)	Claims	1-88	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 1-20 and 51-70 lack an inventive step under PCT Article 33(3) as being obvious over Naughton et al in view of Sussman et al and Stephanopoulos et al.

Claims 1-20 are drawn to a method of expanding/maintaining undifferentiated hemopoietic stem cells or progenitor cells by seeding the stem cells into a stationary phase plug-flow bioreactor in which a three-dimensional stromal cell culture has been pre-established on a non-woven fibrous matrix in the form of a sheet, and expanding/maintaining the undifferentiated hemopoietic stem cells or progenitor cells.

Claims 51-70 require a method of transplanting undifferentiated hemopoietic stem cells or progenitor cells resulting from expanding/maintaining the cells by the method of claims 1-20.
Naughton et al disclose growing stromal cells on a three-dimensional matrix which can be formed from a polymeric material (column 10, lines 55-67) to produce a three-dimensional stromal matrix (column 9, lines 16-20 and 49-51 and column 13, lines 8-14), inoculating the stromal matrix with stem cells (column 15, lines 41 and 57 and column 21, lines 3, 9 and 26) such as hematopoietic stem cells (column 21, line 3), maintaining the stem cells on the matrix *in vitro* where proliferation of the cells is maximized (column 21, lines 2-3), and implanting the stem cells *in vivo* to repopulate bone marrow (column 16, lines 58-67 and column 21, line 4-5).

Sussman et al disclose a fibrous matrix for cell cultivation. The matrix can be a non-woven fiber sheet (column 4, line 56), and can have a pore volume of 40-90%, a pore size of 10-100 μm, a height of 50-500 μm and a fiber diameter of 0.5-50 μm (column 2, lines 47-65). Matrix sheets can be used as a packing in a column (paragraph bridging columns 7 and 8), and the matrix can be coated with poly-D-lysine (column 13, line 68).

Stephanopoulos et al disclose a cell-culturing reactor having an inlet and outlet for culture medium and containing a macroporous (Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:
The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(7): C12N 5/00, 5/06, 11/02, 11/08; A61F 2/02; C12M 3/00, 3/04 and US Cl.: 424/93.7, 423; 435/177, 180, 347, 373, 395, 398, 289.1

I. BASIS OF REPORT:

This report has been drawn on the basis of the description,
page(s) 1-43, as originally filed.
page(s) NONE, filed with the demand.
and additional amendments:
NONE

This report has been drawn on the basis of the claims,
page(s) 44-58, as originally filed.
page(s) NONE, as amended under Article 19.
page(s) NONE, filed with the demand.
and additional amendments:
Page 59 filed with the letter of 29 May 2001.

This report has been drawn on the basis of the drawings,
page(s) 1-4, as originally filed.
page(s) NONE, filed with the demand.
and additional amendments:
NONE

This report has been drawn on the basis of the sequence listing part of the description:
page(s) NONE, as originally filed.
pages(s) NONE, filed with the demand.
and additional amendments:
NONE

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):
support between the inlet and outlet having pores of a size that allows cells to collect within the pores and oxygen and nutrients to migrate into the pores for consumption by the cells (paragraph bridging columns 2 and 3).

It would have been obvious to use as the matrix of Naughton et al the non-woven fibrous sheet packed in a column for cell culture disclosed by Sussman et al to obtain a flow through reactor having an inlet and outlet as suggested by Sussman et al and Stephanopoulos et al since such a reactor would have been expected to provide advantages of a beneficial environment for cell culture and continuous flow.

In the response of 29 May 2001, applicants urge that Naughton et al is maintaining a differentiated cell population, and fails to teach or suggest expanding and maintaining undifferentiated stem cells.

This argument is unpersuasive since the description of Naughton et al indicates that an embodiment is to expand and maintain undifferentiated stem cells. See column 21, lines 1-7, where Naughton et al disclose that proliferation of multipotential hematopoietic stem cells is maximized. Further see lines 26-28 of column 21 where it is further disclosed that stem cell replication can be inferred from the sustained proliferation of committed progenitors. It is clear from this disclosure in column 21 that Naughton et al intend to expand and maintain stem cells while the cells are undifferentiated, and is not expanding and maintaining only a differentiated cell population. Moreover, it would have been obvious to use the procedure of Naughton et al to expand and maintain undifferentiated stem cells to provide undifferentiated stem cells for implanting so that the stem cells can differentiate *in vivo*. Implanting of stem cells for differentiation *in vivo* is well known.

Applicants urge that Figure 1 in the description shows that using a plug-flow reactor as claimed supports a higher percentage of seeded stem cells than the use of static culture as in Naughton et al. However, the claims do not require a procedure of

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

using a plug-flow reactor commensurate in scope with that used to obtain the results shown by Figure 1, and the claimed procedure would not have to produce a higher percentage of stem cells supported. Moreover, Sussman et al and Stephanopoulos et al clearly suggest using a plug-flow reactor for culturing in Naughton et al to provide continuous operation and a suitable environment for cell cultivation, and an increased percentage of supported stem cells would have been inherent. Discovering an additional result of supporting an increased percentage of seeded stem cells does not make unobvious the use of a plug-flow reactor for the reason suggested by Sussman et al and Stephanopoulos et al.

Claims 21-50 and 71-88 lack an inventive step under PCT Article 33(3) as being obvious over the prior art as applied in the immediately preceding paragraph and further in view of Emerson et al.

Claims 21-50 require a method of expanding/maintaining undifferentiated hemopoietic stem cells or progenitor cells by culturing the cells in a stromal cell conditioned medium derived from a stationary phase plug-flow bioreactor in which a three-dimensional stromal cell culture has been established on a non-woven fibrous matrix in the form of a sheet.

Claims 71-88 require a method of transplanting undifferentiated hemopoietic stem cells or progenitor cells resulting from expanding/maintaining the cells by the method of claims 21-50.

Emerson et al disclose stem cell expansion in a stromal cell conditioned medium.

When using a non-woven fibrous sheet in a flow through reactor as the matrix to form the stromal matrix of Naughton et al for culturing stem cells as set forth above, it would have been obvious to use the stromal matrix to form a conditioned medium and culture the stem cells in the conditioned medium as suggested by Emerson et al.

Applicants urge that it is unclear where Emerson et al disclose stem cell expansion in a stromal cell conditioned medium. This is disclosed at column 7, lines 55-59, where Emerson et al disclose that stomal cells may be present in cultures. The presence of stromal cells in the medium used to culture stem cells would have resulted in a stromal cell conditioned medium.

It is granted as urged by applicants that Emerson et al teach 50-100% medium replacement daily, and add growth factors to the medium. However, the present claims do not exclude medium replacement and addition of growth factors as taught by Emerson et al. Moreover, it would have been obvious to omit medium replacement if the result of removing metabolic products and replenishing depleted nutrients as disclosed by Emerson et al is not desired. Furthermore, the addition of growth factors by Emerson et al is to provide optimal growth conditions, and omitting the growth factors would have been obvious if optimal growth conditions are not desired.

Claims 1-88 meet the criteria set out in PCT Article 33(2), because a single prior art reference does not teach or fairly suggest the claimed invention.

Claims 1-88 meet the criteria set out in PCT Article 33(4), because the claimed invention has utility, and therefore has industrial applicability.

---------- NEW CITATIONS ----------
NONE

88. The method of claim 71, wherein the matrix is coated with poly-D-lysine.